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DATA EVALUATION RECORD

STUDY TYPE: Rodent *In Vivo* Dermal Penetration Study – Rat
OPPTS 870.7600 [§85-2]; OECD none.

PC CODE: 025002 **DP BARCODE:** 339369

TEST MATERIAL (PURITY): Creosote (98.5%)

SYNONYMS: AWPA P1-P13 Creosote; American Wood Preserves Association P1-P13 Creosote

CITATION: Fasano, W. (2007) AWPA P1-P13 Creosote: In vivo dermal absorption in the rat. E.I. du Pont de Nemours and Company, Haskell[SM] Laboratory for Health and Environmental Sciences, Newark, Delaware. Laboratory Project ID: DuPont-19622, July 2, 2007. MRID 47179501. Unpublished.

SPONSOR: The Creosote Council III, P.O. Box 160, Valencia, Pennsylvania 16059.

EXECUTIVE SUMMARY: In a dermal penetration study (MRID 47179501) AWPA P1-P13 Creosote (98.5% a.i.) spiked with eight radiolabeled target chemicals (approx. 43% of the chemicals in creosote) was applied to the clipped dorso-lumbar skin (10.5 cm^2) of eight male Sprague-Dawley rats at a dose of 10.7 mg/cm^2 skin (10 $\mu L/cm^2$, total radioactivity 16.7 μCi). The exposure duration was 8 hours. At the end of 8 hours, animals were removed from the metabolism cages, and the application sites were washed with 2% Ivory soap solution followed by a water rinse and then a dry natural sponge. Four rats were then euthanized (0 hours post-exposure) while the other 4 rats were returned to the metabolism cages for further excreta collection. These 4 rats were sacrificed at 496 hours post-exposure.

The mean percent recovery of the radioactivity in the applied dose was 95.009% (±2.569%) and 96.609% (±3.628%) at 0 and 496 hours post-exposure, respectively. The majority of the administered dose was recovered from the skin wash sponges; the mean percent recovery was 59.283% (±12.54%) for the 0-hr post-exposure group and 56.822% (±8.294%) for the 496-hr post-exposure group. The mean percent absorbed dose was 6.342% (±0.808%) at 0 hours post-exposure and 33.959% (±8.445%) at 496 hours post-exposure, with a mean percent of 1.553% (±0.299%) and 0.005% (±0.003%), respectively, present in the skin after removal of the stratum corneum. Most of the absorbed dose had been excreted by 496 hours post-exposure, with a mean of 18.97% (±4.97%) of the dose eliminated in the urine and 12.60% (±4.11%) eliminated in the feces.

In a supplemental dermal penetration study intended to clarify the results for the 496 post-exposure group in the initial main study, AWPA P1-P13 Creosote (98.5% a.i.) spiked with eight radiolabeled target chemicals was applied to the shaved dorsal skin (10.5 cm^2) of four male Sprague-Dawley rats at a dose of 10.7 mg/cm^2 skin (10 $\mu L/cm^2$, total radioactivity 18.9 μCi). The exposure duration was 8 hours. Animals were sacrificed at 496 hrs post-exposure (504 hrs post-dosing). In the supplemental absorption study, the O-ring, which demarcated the application area, was applied without glue following the dose application, and was removed at the end of the 8-hr exposure. In the main study, the O-ring had been glued to the skin one day prior to dose application and remained in place until study termination.

In the supplemental study, the mean percent recovery of the radioactivity in the applied dose was 92.775% (±2.70%) at 496 hours post-exposure. The majority of the administered dose was recovered from the body wrap (34.228±6.328%), skin wash (22.240±7.818%), and O-ring (27.452±3.680%). Although the mean percent absorbed dose was reported in this supplemental experiment as 8.853±1570%, with a mean percent of 0.005% present in the skin after removal of the stratum corneum, the presence of a significant amount of radioactivity in the body wrap after 8 hours adds uncertainty as to the actual available dose. In addition, in the first experiment, dermal absorption was still evident after washing of the skin at 8 hours and monitoring of urinary and fecal excretion of radioactivity from 8 hours onward. Therefore, the data are not conclusive of a reduced dermal absorption based on the contention that the O-ring or the glue had an influence on dermal absorption.

In a preliminary plasma kinetic (dermal bioavailability) study, neat creosote was applied to the clipped dorso-lumbar skin (10.5 cm^2) of four male Sprague-Dawley rats (10 $\mu L/cm^2$) for an exposure duration of 8 hours; animals were sacrificed 168 hours post-dosing (160 hours post-exposure). None of the twelve target creosote chemicals analyzed in the plasma were detected above their detection limit.

This study in the rat is **ACCEPTABLE-NON-GUIDELINE** Although only one exposure duration and one exposure concentration were evaluated, this was part of an agreement in study design between the Antimicrobials Division and the registrant.

COMPLIANCE: Signed and dated No Data Confidentiality, GLP, and Quality Assurance statements were included. The GLP statement notes the following exception to compliance with 40 CFR Part 160: the chemical and radiochemical concentration and the radiochemical purity of the selected chemicals of the creosote test substance that was spiked with radiolabeled chemicals was based on the certificates of analyses provided by the sponsor and vendors and the verified radioactivity per volume.

I. MATERIALS AND METHODS:

A. MATERIALS:

1.	**Test material:**	AWPA P1-P13 Creosote
	Description:	Creosote: dark, amber-colored liquid Radiolabeled chemicals: benzo(a)pyrene (toluene solution); 2-methylnaphthalene (solid); fluoranthene (methanol solution); anthracene (toluene solution); naphthalene-benzene (solid); phenanthrene (solid); biphenyl (toluene solution); pyrene (solid)
	Lot/batch #:	Creosote test substance: not provided Radiolabeled chemicals: benzo(a)pyrene (033H9241); 2-methylnaphthalene (050K9424/25); fluoranthene (054K9630); anthracene (018H9432/33); naphthalene-benzene (068H9600/01); phenanthrene (111K9412/13); biphenyl (115F9247); pyrene (079H9662/63)
	Purity:	Creosote: 98.5% a.i. Radiolabeled chemicals: 2-methylnapthalene (99.707%); others (not provided)
	Compound stability:	Minimum of 4 years (storage conditions not specified)
	CAS # for TGAI:	8001-58-9
	Structure:	Not applicable; a mixture of aromatic hydrocarbons
	Vehicle/Solvent used:	Not applicable; test substance applied as neat creosote
	Radiolabeling:	benzo(a)pyrene-7-^{14}C; 2-methylnaphthalene- 8-^{14}C; fluoranthene-3-^{14}C; anthracene-1,2,3,4,4A,9A-^{14}C; naphthalene-benzene-UL-^{14}C; phenanthrene-9-^{14}C; biphenyl-UL-^{14}C; pyrene-4,5,9,10-^{14}C
	Specific Activity:	benzo(a)pyrene (26.6 mCi/mmol); 2-methylnaphthalene (8.5 mCi/mmol); fluoranthene (45 mCi/mmol); anthracene (20.6 mCi/mmol); naphthalene-benzene (31.3 mCi/mmol); phenanthrene (8.2 mCi/mmol); biphenyl (7.6 mCi/mmol); pyrene (55 mCi/mmol)
	Radiochemical Purity:	benzo(a)pyrene (94.781%); 2-methylnaphthalene (not provided);

	fluoranthene (99.523%); anthracene (97.524%); naphthalene-benzene (99.119%); phenanthrene (99.623%); biphenyl (100%); pyrene (97.186%)
Source:	Creosote: sponsor Selected radiolabeled chemicals: Sigma-Aldrich Co., St. Louis MO

2. **Relevance of test material to proposed formulation(s):** In the main and supplemental *in vivo* dermal absorption studies, the creosote test material was applied neat, spiked with eight selected radiolabeled chemicals. In the plasma kinetic study, the creosote test material was applied undiluted (neat).

3. **Test animals:**

Species:	Rat, male
Strain:	Sprague-Dawley Crl:CD(SD)
Age/weight at study initiation:	8-10 weeks; 313.4 to 362.4 grams
Source:	Charles River Laboratories (Raleigh, NC)
Housing:	Individually in all-glass metabolism cages (source not provided)
Diet:	PMI® Nutrition International, LLC Certified Rodent LabDiet® 5002, *ad libitum*; feed certified by supplier not to exceed maximum concentrations of key contaminants
Water:	Tap water, *ad libitum*; water samples analyzed periodically for coliform bacteria, lead, and other contaminants
Environmental conditions:	**Temperature:** 18-26°C **Humidity:** 30-70% **Air changes:** Not provided **Photoperiod:** 12 hrs dark/ 12 hrs light (fluorescent light)
Acclimation period:	Plasma kinetic study and main dermal absorption study: fitted with O-ring one day prior to dosing and acclimated overnight in individual all-glass metabolism cages Supplemental dermal absorption study: acclimated overnight in individual all-glass metabolism cages
Comments:	animals used in the plasma kinetic study were obtained from the supplier with a cannula inserted in the jugular vein

B. STUDY DESIGN: Three preliminary studies were conducted prior to the main *in vivo* dermal absorption study: a general abundance study, a study to develop a quantitative extraction method for creosote chemicals in rat plasma, and a plasma kinetic (bioavailability) study. Following the main dermal absorption study, a supplemental dermal absorption study was conducted to clarify the results of the initial 496-hr post-exposure group.

The objective of the general abundance study was to assess the types of chemicals present in creosote. The relative concentrations of the following twelve target chemicals were determined using gas chromatography with mass spectrometry detection (GC-MS): 1-methylnapthalene, 2-methylnaphthalene, acenaphthalene, benzo(a)pyrene, benzo(b)fluoranthene, carbazole, dibenzofuran, fluoranthene, fluorene, naphthalene, phenanthrene, and pyrene. The abundance of each chemical was estimated by total mass

spectral response and the results compared with the certificate of analysis provided by the sponsor. The identification of each chemical was confirmed by automatic spectral comparison with the Wiley Registry of Mass Spectral Data.

The objective of the second preliminary study was to develop a quantitative extraction method from rat plasma for the twelve creosote chemicals of interest. Rat plasma was fortified with creosote test substance in dimethyl sulfoxide at four concentrations (concentrations not provided). The plasma was spiked with 500 µL of acetonitrile and vortexed to precipitate out plasma proteins. Following protein precipitation, anhydrous sodium sulfate (200 mg), toluene (990 µL), and phenanthrene-d_{10} in toluene internal standard solution (10 µL of 150 µg/mL) were added, and the mixture vortexed and centrifuged. The organic layer was separated and then analyzed by GC-MS.

The third preliminary study (plasma kinetic study) was an *in vivo* dermal study designed to establish the bioavailability of selected creosote chemicals, and to provide the rationale for the selection of the radiolabeled chemicals used in the main *in vivo* dermal absorption study.

The designs for the *in vivo* plasma kinetic study and the main and supplemental *in vivo* dermal absorption studies are as follows:

1. **Dose:**

Rationale:

Plasma kinetic study, and main and supplemental dermal absorption studies: The exposure time and application rates were intended to mimic potential exposures to neat creosote.

Nominal doses:

Plasma kinetic study: Not provided

Main and supplemental dermal absorption studies: The application amounts and rates are summarized in Table 1.

Table 1: Dosing (mean±SD) – Main and Supplemental Dermal Absorption Studies[a]

Weight of Formulation (grams)[b]	Total Radioactivity Applied (µCi)	Total Creosote Applied (µg)[c]	Application Rate (µg/cm^2 skin)[d]
Main Study			
0.1124±0.000	16.7±0.00	112350±0.00	10700±0.00
Supplemental Study			
0.1124±0.000	18.9±0.14	112350±0.00	10700±0.00

[a] Data and formulas were obtained from page 99 (Appendix C) of the study report.

[b] application volume of 105 µL x density of 1.07 grams/mL = 0.11235 grams applied

[c] weight applied x 1,0000,000 µg/gram

[d] application rate = total creosote applied (112350 µg) / 10.5 cm^2

Actual doses:

Plasma kinetic study, and main and supplemental dermal absorption studies: Not provided

Dose volume:

Plasma kinetic study, and main and supplemental dermal absorption studies: 10 $\mu L/cm^2$ skin

Duration of exposure (time from dose to skin wash):

Plasma kinetic study, and main and supplemental dermal absorption studies: 8 hours

Termination periods (time from dose to sacrifice):

Plasma kinetic study: 168 hours post-dose (all animals)

Main dermal absorption study: 8 hours post-dose (0-hr post-exposure group, 4 animals) or 504 hours post-dose (496-hr post-exposure group, 4 animals)

Supplemental dermal absorption study: 504 hours post-dose (496-hr post-exposure group, all animals)

Number of animals/group:

Plasma kinetic study: 4 (one dose group)

Main dermal absorption study: 8 (one dose group)

Supplemental dermal absorption study: 4 (one dose group)

2. **Animal preparation:**

Plasma kinetic study and main dermal absorption study: One day before dosing, the animals were anesthetized with isofluorane®, the back and shoulders clipped free of hair, and the clipped area washed with aqueous 2% Ivory® Soap solution. A glass O-ring with an internal diameter of 10.5 cm^2 was glued to the clipped area on the back with Instant Krazy Glue Gel adhesive, and the O-ring covered with Coban™ body wrap (porous). The rats were then acclimated overnight in individual all-glass metabolism units.

Supplemental dermal absorption study: One day before dosing, the animals were anesthetized with isofluorane®, the dorsal area shaved, and an area of 10.5 cm^2 demarcated within the shaved area using indelible ink. The shaved and demarcated area was protected with Coban™ body wrap and the animals acclimated overnight in individual all-glass metabolism units.

3. Dose preparation, administration and quantification:

Preparation:

Plasma kinetic study: The creosote test substance was used neat as provided by the sponsor.

Main and supplemental dermal absorption studies: Eight radiolabeled substances, representing approximately 43% of the chemicals in creosote, were selected for spiking the creosote test substance. Toluene was added to each of these materials and the amount of radioactivity per volume verified by analysis of representative aliquots using liquid scintillation counting (LSC). Based on these results and the target level of activity, summarized in Table 2, aliquots of each were removed and combined in a vial. The sample was evaporated to dryness by nitrogen convection (with an isopropyl alcohol trap) and approximately 5 mL of creosote was added. The substances were mixed and sonicated.

Table 2: Target Activity Levels for Key Radiolabeled Chemicals[a]

Radiolabeled Chemical	Concentration in Creosote (%)[b]	Amount of Chemical in 5 mL	Specific Activity of Neat Sample (µCi/mg)	Amount of Activity Required (µCi)
Phenanthrene	12.2	653	46.1	250
Naphthalene	9.0	482	244.5	184
Fluoranthene	6.8	364	222.8	139
Pyrene	6.0	321	272.3	123
2-Methylnaphthalene	5.1	273	59.9	105
Anthracene	2.2	118	115.7	45
Biphenyl	1.2	64	49.4	25
Benzo(a)pyrene	0.5	27	105.6	10
	43.0 (Total)			

[a] Data were obtained from page 20 of the study report.
[b] Based on the certificate of analysis provided by the sponsor.

The final specific activity of each of the eight radiolabeled chemicals was based on the specific activity of each neat chemical (supplier's certificate of analysis), the amount of radioactivity of each added to creosote, and the nominal concentration of each chemical in the creosote test substance (sponsor's certificate of analysis). The calculated nominal specific activity target for each of the eight radiolabeled chemicals was 0.39 µCi/mg, providing equivalent radiochemical sensitivity for each.

The application amounts and rates are summarized in Table 1.

The length of time between the preparation and application of the test substance was not provided.

Application:

Plasma kinetic study: On the day of dosing, the Coban™ body wrap was removed and the

test substance applied at 10 μL/cm^2 to the shaved dorsal skin. The animals were then returned to individual all-glass metabolism cages (brand not specified).

Main dermal absorption study: On the day of dosing, the Coban™ body wrap was removed and the test substance applied at 10 μL/cm^2 to the shaved dorsal skin. A glass cap containing Anasorb® 747 trapping medium (SKC Inc., Eighty-Four, PA) was fitted onto the top of the glass O-ring and the glass appliance secured with Coban™ body wrap. The animals were returned to individual all-glass metabolism cages suitable for the collection of $^{14}CO_2$, ^{14}C-volatile organics, urine, and feces (brand not specified).

Supplemental dermal absorption study: On the day of dosing, the Coban™ body wrap was removed and the test substance applied at 10 μL/cm^2 within the demarcated area. Following dosing, silicone O-ring spacers were placed over the dose site without glue and wrapped securely with Coban™. An organic volatile trap was not used because <0.5% of the applied dose (on average) evolved from the dose site in the main dermal absorption study.

Quantification:

Plasma kinetic study: Not provided

Main and supplemental dermal absorption studies: The amount and distribution (homogeneity) of radioactivity per volume of the spiked test substance was determined by analyzing three 105 μL aliquots (volume of applied dose) by LSC. The results were not provided.

4. Skin wash (pre-sacrifice):

Plasma kinetic study: At 8 hours post-dosing, the application site was cleansed with a minimum of three wash, rinse, and dry cycles as follows: one natural sponge soaked in 2% Ivory® Soap, followed by one sponge soaked with water, followed by one dry natural sponge. Animals were returned to their metabolism cages until sacrifice (168 hours post-dosing).

Main dermal absorption study: At 8 hours post-dosing, each rat was removed from the metabolism cage and the body wrap and volatile organic trapping contents removed. The application site was washed with 2% Ivory® Soap solution. Four of the rats were euthanized following the wash. The surviving rats had a fresh volatile organic trap and body wrap applied and were returned to their metabolism cages until sacrifice at 504 hours post-dosing.

Supplemental dermal absorption study: At 8 hours post-dosing, the body wrap and silicone O-ring spacers were removed, the skin washed with a 2% soap solution, and the rats wrapped with a fresh Coban™ body wrap to protect the dose site. The rats were returned to their metabolism cages until sacrifice at 504 hours post-dosing.

5. Sample collection:

Plasma kinetic study: Whole blood samples (100 μL) were collected pre-dose; post-dose at

0.5, 1, 2, 4, 6, 8, 10, 22, and 24 hours; and then at 24-hr intervals until sacrifice. Whole blood for each of two rats was pooled.

Main dermal absorption study: Urine and feces were collected in vessels cooled by solid carbon dioxide during the 8-hr exposure period. For the surviving animals, urine and feces were collected for 8-12 and 12-24 hour intervals post-dosing, and then at 24-hr intervals until sacrifice.

Residual feed and cage washings were collected, as needed.

The closed system chamber air was drawn serially through a 2N NaOH trap to collect $^{14}CO_2$ and through an ethylene glycol trap to collect ^{14}C-volatile organics. Samples were collected during the 8-hr exposure period for all test animals. For the surviving animals, samples were collected for 8-12 and 12-24 hour intervals post-dosing, and then at 24-hr intervals until radioactivity in sample aliquots was less than or equal to the limit of detection.

The charcoal trap medium was collected at 8 hours post-dosing for the 0-hr post-exposure group and at 216 hours post-dosing for the 496-hr post-exposure group. Because stress was apparent at 216 hours post-dosing, as evidenced by decreased body weight, the glass cap of the charcoal trap was removed at 216 hours, rather than remaining in place until sacrifice. *(Note: It is stated on page 21 of the study report that the charcoal trap was removed 216 hours post-exposure. This is likely an error. The last data set for the charcoal trap is for 216 hours post-dosing; page 105 of the study report).*

Sponge pieces used to wash the application site were retained.

Body wrap and the organic trapping medium were collected 8 hours post-dosing for all animals, and at sacrifice for the surviving animals. The O-ring appliance was removed at the time of sacrifice and retained. After sacrifice, the application site skin was tape-stripped to remove the stratum corneum and the tape and skin retained. The heart, lungs, kidneys, and liver were excised, held briefly on wet ice, and stored.

The body wrap, organic volatile trapping media, glass O-rings, and tissues were removed while the animals were under isofluorane® anesthesia. Animals were sacrificed while anesthetized by cardiac puncture exsanguination, and whole blood collected.

Supplemental dermal absorption study: Urine and feces were collected for 0-8 hour and 8-24 hour intervals post-dosing, then at 24-hr intervals until sacrifice. The Coban™ body wrap was changed periodically over the course of the 496-hr post-exposure period at the discretion of the Study Director. $^{14}CO_2$ and ^{14}C-volatiles were not collected because <0.3% of the applied dose (on average) was collected from these samples in the main dermal absorption study (slightly less than <0.5% for volatile organics, below the limit of detection for CO_2). At the end of the 496-hr post-exposure period, the rats were processed for a complete mass balance of the applied dose as previously described for the main dermal absorption study.

6. Sample preparation and analysis:

Plasma kinetic study: Whole blood was held on wet ice and the plasma separated from the red cell fraction by centrifugation. Plasma samples (100 µL) were immediately prepared for analysis by adding 500 µL of acetonitrile and vortexing to precipitate out plasma proteins. Following protein precipitation, anhydrous sodium sulfate (200 mg), toluene (990 µL), and phenanthrene-d_{10} in toluene internal standard solution (10 µL of 150 µg/mL) were added, and the mixture vortexed and centrifuged. The organic layer was separated and then analyzed by GC-MS.

Main and supplemental dermal absorption studies: Samples not immediately processed for analysis were stored at #-10°C (plasma, carcasses, urine, feces, application site skin, tissue) or refrigerated at approximately 0-10°C (cage wash, residual feed, whole blood, red blood cells, sponge pieces, body wrap, O-rings, tape strips).

Urine, cage washings, NaOH ($^{14}CO_2$ trapping medium, main study only), and ethylene glycol (^{14}C-volatile organic trapping medium, main study only) were not processed further. Aliquots of these sample types were added directly to liquid scintillant (e.g., Ultima Gold™ XR). Additional details of sample preparation for other sample types are provided in Table 3.

TABLE 3: Sample Preparation Details

Sample Type	Preparation Details
Blood	Whole blood was centrifuged to obtain plasma and red blood cell fractions. Aliquots of whole blood and red blood cells were combusted prior to determination of radioactivity. Aliquots of plasma were added directly to liquid scintillant (e.g., Ultima Gold™ XR).
Residual feed	Residual feed was homogenized in water and aliquots combusted.
Carcass	Carcasses were homogenized with water and aliquots combusted.
Sponge pieces	Sponge pieces were digested in Soluene®-350 and aliquots added to Hionic-Fluor™ liquid scintillant.
Volatile organics trapping medium	The trapping medium was extracted with acetonitrile and aliquots added to Ultima Gold™ XR liquid scintillant.
Body wrap	The body wrap was extracted with acetonitrile and aliquots added to Ultima Gold™ XR liquid scintillant.
O-rings	The O-rings were extracted with acetonitrile and aliquots added to Ultima Gold™ XR liquid scintillant.
Tape strips	The tape strips were extracted with acetonitrile and aliquots added to Ultima Gold™ XR liquid scintillant.
Skin at application site	The skin at the application site was excised and tape-stripped using Leukotape® P (BSN Medical, Ltd., Pinetown, South Africa) to remove the stratum corneum. The tape strips were placed in individual glass vials and extracted with acetonitrile. The application site skin was placed in a glass container. Skin tissue was digested in Soluene®-350. Aliquots were added directly to Hionic-Fluor™ liquid scintillant.
Heart	The tissue was minced and aliquots combusted.
Lungs	The tissue was minced and aliquots combusted.
Kidney	The tissue was minced and aliquots combusted.

TABLE 3: Sample Preparation Details	
Sample Type	**Preparation Details**
Liver	The tissue was minced and aliquots combusted.

Aliquots of whole blood, red blood cells, feces, residual feed, carcass homogenate, and tissues were combusted using a Packard Tri-Carb Automatic Sample Oxidizer. The $^{14}CO_2$ generated was collected in an absorbent scintillation system.

All samples were analyzed for total radioactivity in a Packard liquid scintillation counter. Samples were counted for 10 minutes or until 160,000 disintegrations were accumulated. The limit of detection and the limit of quantitation for each sample analysis were taken, respectively, as twice and three times the background disintegration rate obtained from analysis of appropriate blank samples.

Calculated values were generated by computer and by Debra (V.5.1a), a protocol-driven GLP-compliant laboratory information system (LabLogic Systems Ltd., Sheffield, England).

The total amount of radioactivity in samples was reported as a percentage of the total dose.

II. RESULTS:

For the general abundance study, GC-MS analysis indicated that all twelve target chemicals analyzed in the creosote test substance were well-resolved. The data were presented as a chromatogram (page 44 of the study report).

The results of the second preliminary study, which evaluated the recovery of the twelve target chemicals from spiked rat plasma, indicated average recoveries ranging from approximately 66% to 106%, with the exception of benzo(a)pyrene (approx. 54%). When extracted from frozen plasma, the recovery of these chemicals was >80%. The study result data were not provided.

The results of the plasma kinetic study, and the main and supplemental *in vivo* dermal absorption studies are as follows:

A. SIGNS AND SYMPTOMS OF TOXICITY:

Plasma kinetic study: No signs or symptoms of toxicity were reported.

Main dermal absorption study: Toxicity, as evidenced by decreased body weight, was reported for the 496-hr post-exposure group at 216 hours post-dosing. These body weight data were not provided. The body weight data included in the report were limited to body weights at the initiation of the study.

Supplemental dermal absorption study: No signs or symptoms of toxicity were reported.

B. SUMMARY TABLES:

Plasma kinetic study: The detection and quantitation limits for the twelve target chemicals analyzed in rat plasma are summarized in Table 4.

Table 4: Detection and Quantitation Limits for Twelve Creosote Target Chemicals[a]

Target Analyte	Limit of Detection (ppm)	Limit of Quantitation (ppm)
Naphthalene	0.120	0.400
2-Methylnaphthalene	0.027	0.090
1-Methylnaphthalene	0.020	0.067
Acenaphthene	0.016	0.053
Dibenzofuran	0.009	0.030
Fluorene	0.043	0.143
Phenanthrene	0.135	0.450
Fluoranthene	0.181	0.603
Pyrene	0.174	0.508
Carbazole	0.022	0.073
Benzo(b)fluoranthene	0.090	0.300
Benzo(a)pyrene	0.056	0.187

[a] Data were obtained from page 33 (Table 2) of the study report.

Main and supplemental dermal absorption studies: The mean percent recovery of radioactivity by sample type is summarized in Table 5.

Table 5: Percent Recovery of Applied Dose for Each Sample Type[a]

Sample Type	Percent of Administered Dose (mean±SD)		
	Main Study 0 Hours Post-Exposure (n=4)	Main Study 496 Hours Post-Exposure (n=4)	Supplemental Study 496 Hours Post-Exposure (n=4)
Urine	2.129±0.360	18.967±4.973	4.812±0.862
Feces	0.026±0.026	12.604±4.105	3.423±0.939
Cage wash	0.697±0.272	1.670±1.131	0.551±0.137
Carbon dioxide	NA	0.000±0.000	NS
Residual feed	0.005±0.001	0.241±0.174	0.066±0.076
Volatile organics	0.049±NA	0.233±0.224	NS
Non-dosed skin	0.020±0.004	0.039±0.014	0.001±NA
Carcass	3.144±0.524	0.195±0.211	NA
Whole blood	0.027±0.006	0.003±0.001	0.001±NA
Red blood cells (terminal)	0.011±0.004	0.002±0.001	0.001±0.001
Heart	0.002±0.000	NA	NA
Lungs	0.006±0.002	NA	NA
Liver	0.185±0.015	0.005±0.002	NA
Kidney	0.073±0.009	0.002±0.001	<0.001±NA
Plasma (terminal)	0.011±0.002	NA	NA
Total Absorbed Dose	**6.342±0.808**	**33.959±8.445**	**8.853±1.570**
Absorbed dose	6.342±0.808	33.959±8.445	8.853±1.570
Absorbable dosed skin	1.553±0.299	0.005±0.003	0.005±NA
Total Dose Absorbable	**7.895±1.081**	**33.964±8.447**	**8.855±1.570**

Table 5: Percent Recovery of Applied Dose for Each Sample Type[a]			
Sample Type	Percent of Administered Dose (mean±SD)		
	Main Study 0 Hours Post-Exposure (n=4)	Main Study 496 Hours Post-Exposure (n=4)	Supplemental Study 496 Hours Post-Exposure (n=4)
Body wrap	2.097±2.186	2.443±1.437	34.228±6.328[b]
Skin wash – sponges	59.283±12.547	56.822±8.294	22.240±7.818
Charcoal trap	0.490±0.152	0.451±0.108	NS
O-ring	18.357±8.177	2.889±0.511	27.452±3.680
Tape strips	6.886±2.744	0.041±0.040	0.001±0.002
Total Unabsorbed Dose	**87.113±3.545**	**62.645±8.404**	**83.920±2.308**
Total Dose Recovered	**95.009±2.569**	**96.609±3.628**	**92.775±2.700**

[a] Data and qualifiers were obtained from page 35 (Table 4) and pages 102 and 110 (Appendix C) of the study report.
[b] Approximately 32% of the applied dose was collected at the end of the 8-hr period and the balance (approximately 2%) was collected post-exposure.
NA – Not applicable. Samples were below the limit of detection or limit of quantitation.
NS – No samples collected.

C. TOTAL ABSORBED DOSE:

Plasma kinetic study: A total absorbed dose was not calculated. None of the twelve target chemicals were detected in plasma at or above the limit of detection. The detection and quantitation limits for the target chemicals are summarized in Table 4.

Main dermal absorption study: The mean total absorbed dose (as a percent of the applied dose) was calculated as the sum of the mean percent radioactive doses detected in urine, feces, cage wash, residual feed, carcass, tissues, whole blood, red blood cells, plasma, expired air, and trapped volatile organics. The mean total absorbable dose was considered to be the sum of the mean percent absorbed dose plus the mean percent radioactivity from the tape strips.

The results of the sample analyses are summarized in Table 5, reproduced from the study report. The recovery of the applied dose (mass balance) was acceptable. The mean percent recovery was approximately 95.0% for the 0-hr post-exposure group and 96.6% for the 496-hr post-exposure group. The results were not adjusted for incomplete recovery of the applied dose.

The majority of the administered dose was recovered from the skin wash sponges (approx. 59.3% and 56.8% for the 0-hr and 496-hr post-exposure groups, respectively).

For the 0-hr post-exposure group, the mean total absorbable dose after 8 hours of exposure was approximately 15.6% (total dose absorbed + dosed skin + tape strips). For the 496-hr post-exposure group, both the mean total absorbable dose and mean total absorbed dose were approximately 34% after 8 hours of exposure, with only a very small amount (0.005%) remaining in the skin after tape-stripping. Most of the absorbed dose was eliminated by 496 hours post-exposure, with approximately 31.6% of the applied dose being found in the urine and feces combined. It is noted that urinary and fecal excretion of dermally applied

radioactivity continued beyond the 8 hour time point in those animals which had skin washes at 8 hours and were continued to be monitored for dermal absorption beyond 8 hours.

Supplemental dermal absorption study: The total absorbed dose for the supplemental 496-hr post-exposure group study (8.8%) was lower than the main study, as the percent applied dose for the dosed skin and the tape strips was significantly less than in the main study. The results of the sample analyses are summarized in Table 5.

The recovery of the applied dose, approximately 92.8%, was acceptable. The majority of the administered dose was recovered from the body wrap (approx. 34.2%), skin wash (approx. 22.2%), and O-ring (approx. 27.5%). At 496 hours post-exposure, both the mean total absorbable dose and the mean total absorbed dose were approximately 8.9%, with only a very small amount (0.005%) remaining in the skin after tape-stripping. Most of the absorbed dose was eliminated by 486 hours post-exposure, with approximately 8.2% found in the urine and feces combined.

III. DISCUSSION AND CONCLUSIONS:

A. **INVESTIGATOR'S CONCLUSIONS:** Analysis of the creosote test substance by GC-MS indicated that the twelve chemicals of interest were well-resolved by the analytical procedure. The results of the plasma biokinetic study suggest that these chemicals were metabolized upon first pass through the skin and likely to have negligible bioavailability.

In the main *in vivo* dermal absorption study, most of the applied dose (>56%) was removed by skin washing. Following an 8-hr exposure period, total dose absorbed was estimated as 15.6% when summing the results of tissue and excreta analysis, the dosed skin, and the tape strips. At the 21-day collection period (496 hours post-exposure), the total absorbable dose was estimated as 34%, with a negligible portion of the applied dose (0.04%) remaining in the skin following removal of the stratum corneum.

In the supplemental dermal absorption study, the mean total excreted dose at 496 hours post-dose (urine, feces) was 8.24% (±1.61%) of the applied dose (page 42 of the study report, Table 11). Slightly more than half was excreted in the urine (mean of 4.81%±0.86%), with a mean percent of 3.82% (±0.67%) of the applied dose excreted in the first 24 hours post-dosing, and a mean of 4.72% (±4.70%) by 96 hours (page 40 of the study report, Table 9). At 504 hours post-dosing, slightly less than half of the excreted dose (mean of 3.42%±0.94% of the applied dose) was found in the feces. A mean of 1.94% (±0.76%) of the applied dose was excreted in the feces in the first 24 hours post-dosing, with 3.31% (±0.92%) of the applied dose excreted by 96 hours (page 41 of the study report, Table 10). No noticeable additional excretion of the applied dose occurred through the urine and feces after 360 hours post-dose and 216 hours post-dose, respectively (pages 40 and 41 of the study report, Table 9 and Table 10).

B. REVIEWER COMMENTS: The main and supplemental dermal absorption studies are not typical in that they determined the dermal absorption of spiked radiolabeled chemicals that were added to the test substance, rather than radiolabeled components of the test substance itself. The results reflect the percent recovery of the radioactivity contained in the added radiolabeled chemicals.
Although the mean percent absorbed dose was reported in this supplemental experiment as 8.853±1570%, with a mean percent of 0.005% present in the skin after removal of the stratum corneum, the presence of a significant amount of radioactivity in the body wrap after 8 hours adds uncertainty as to the actual available dose. In addition, in the first experiment, dermal absorption was still evident after washing of the skin at 8 hours and monitoring of urinary and fecal excretion of radioactivity from 8 hours onward. Therefore, the data are not conclusive of a reduced dermal absorption based on the contention that the O-ring or the glue had an influence on dermal absorption.

Additional comments are as follows;

The investigator concludes that in the main dermal absorption study, after 21 days (504 hours post-dosing), 0.04% of the applied dose remained in the skin following removal of the stratum corneum. This is not in agreement with the data in Table 4 of the study report (page 35), as summarized above in Table 5. Approximately 0.005% of the dose was found in the dosed skin; however, 0.04% of the dose was found in the non-dosed skin, and another 0.04% in the tape strips (stratum corneum). In the supplemental dermal absorption study, the percent of the applied dose found in the skin wash (22.2%) is closer to ¼ of the total unabsorbed dose (83.9%), than to ⅓ of the unabsorbed dose, as stated by the investigator (22.2 /83.9 =26.5%).

The investigator's conclusions that the study results suggest the target chemicals were metabolized upon first pass through the skin and likely have limited bioavailability, and that exposure to the target substances is primarily to their metabolites are not adequately explained in the text of the report. Metabolites were not measured in the blood, feces, or urine; therefore, any statement of exposure being only to metabolites is unjustified. Elimination was near complete by day 21, but metabolism of the creosote components cannot be confirmed by this study based solely on the lack of quantifiable radioactivity in the blood alone.

In the main dermal absorption study, the mean total excreted dose at 504 hours post-dose (urine, feces, volatile organic charcoal trap) was 31.57% (±7.82%) of the applied dose (page 39 of the study report, Table 8). The highest percentage was excreted in the urine (mean of 18.97%±4.97%), with a mean percent of 7.46% (±1.48%) of the applied dose excreted in the first 24 hours post-dosing, and a mean of 18.09% (±4.70%) by 192 hours (page 36 of the study report, Table 5). At 504 hours post-dosing, a mean of 12.60% (±4.11%) of the applied dose was found in the feces. A mean of only 1.76% (±1.47%) of the applied dose was excreted in the feces in the first 24 hours post-dosing, with 7.14% (±2.63%) of the applied

dose excreted by 72 hours, and 12.03% (±4.03%) by 312 hours (page 37 of the study report, Table 6). Volatile organics were first detected in the charcoal trapping medium in small amounts (mean of 0.082%±0.036% of applied dose) at 24 hours post-dosing, reaching a mean of 0.451 % (±0.108%) by 216 hours post-dosing (pages 104 and 105 of the study report, Appendix C; *note: although the cumulative percent data for the charcoal trap are supposedly summarized on page 38 of the study report, Table 7, this table does not include the data for the 216-hr time point*). The charcoal trap was removed at 216 hours post-dosing because the animals were showing signs of stress. Volatile organic excretion, as determined by the charcoal trapping medium, was relatively low compared with excretion in the urine and feces; removal of the trap would not be expected to affect the study results.

C. STUDY DEFICIENCIES:

Minor deficiencies were:

- The lot/batch number of the creosote test substance was not provided.
- The number of air changes (environmental conditions) for the test animals was not provided.
- The time between test substance preparation and application was not provided.
- Storage conditions for the test substance were not provided.
- Data were not provided to support the conclusions of the spiked rat plasma recovery study (preliminary study).

D. STUDY CLASSIFICATION: This study in the rat is **ACCEPTABLE-NON-GUIDELINE.** Although only one exposure duration and one exposure concentration were evaluated, this was part of an agreement in study design between the Antimicrobials Division and the registrant.